

(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 87309479.1

(51) Int. Cl.⁴: C 07 H 21/04

(22) Date of filing: 27.10.87

(30) Priority: 31.10.86 US 926010

(43) Date of publication of application:
04.05.88 Bulletin 88/18

(54) Designated Contracting States:
AT BE CH DE ES FR GB IT LI LU NL SE

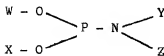
(71) Applicant: AMOCO CORPORATION
200 East Randolph Drive
Chicago Illinois 60601 (US)

(72) Inventor: Groody, Edward Patrick
249 Wilson Street
Marlboro Massachusetts 01752 (US)

(74) Representative: Ritter, Stephen David et al
Mathys & Squire 10 Fleet Street
London EC4Y 1AY (GB)

(54) Compositions and methods for the synthesis of oligonucleotides having 5'-phosphorylated termini.

(57) Compounds of formula



are provided for use in methods for the chemical 5'-phosphorylation of oligonucleotides for DNA phosphoramidite synthesis.

Description

COMPOSITIONS AND METHODS FOR THE SYNTHESIS OF OLIGONUCLEOTIDES HAVING
5'-PHOSPHORYLATED TERMINI

The present invention pertains to compositions, and methods for the use and synthesis of the composition. More particularly, the present invention includes compositions and methods for phosphorylating the 5' termini of oligonucleotides.

Genetic information is stored in living cells in thread like molecules of deoxyribonucleic acid (DNA). In vivo, the DNA molecule is a double helix, each strand of which is a chain of nucleotides. Each nucleotide is characterized by one of four bases: adenine (A), guanine (G), thymine (T), cytosine (C). The bases are complimentary in the sense that due to the orientation of functional groups, certain base pairs attract and bond to each other through hydrogen bonding. Adenine in one strand of DNA pairs with thymine in an opposing complimentary strand. Guanine in one strand of DNA pairs with cytosine in an opposing complimentary strand. In ribonucleic acid (RNA), the thymine base is replaced by uracil (U) which pairs with adenine in an opposing complimentary strand.

DNA consists of covalently linked chains of deoxyribonucleotides and RNA consists of covalently linked chains of ribonucleotides. The genetic code of a living organism is carried upon the DNA strand in the sequence of the base pairs.

Each nucleic acid is linked by a phosphodiester bridge between the 5' hydroxyl group of the sugar of one nucleotide and the 3' hydroxyl group of the sugar of the adjacent nucleotide. Thus, each linear strand of DNA or RNA has one terminal end at a 5' hydroxyl group position and another terminal end at a 3' hydroxyl group position. The terminal ends of polynucleotides are often referred to as being 5' termini or 3' termini in reference to respective free hydroxyl group position. Naturally occur ring DNA and RNA include a phosphate group at the 5' terminal hydroxyl position.

The development of phosphoramidite chemistry has facilitated the routine synthesis of oligonucleotides of a defined sequence. However, most synthetic DNA fragments are isolated with a free 5' hydroxyl group. For many biological applications, it is advantageous to directly isolate DNA having a 5' phosphate group.

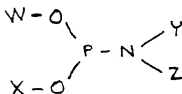
A variety of enzymatic and chemical methods have been developed for the introduction of a 5' phosphate group. Examples of enzymatic or chemical modification of synthetic DNA to introduce a 5' phosphate group are disclosed in Nadeau, J.G., Singleton, C.K., Kelly, G.B., Weith, H.L., and Gough, G.R. (1984) *Biochem.* 23, 6153-6159; van der Marel, G.A., van Boeckel, C.A.A., Wille, G., and van Boom, J.H. (1982) *Nucl. Acids Res.* 10, 2337-2351; Gough, G.R., Bruden, M.J., Nadeau, J.G., and Gilham, P.T. (1982) *Tetrahedron Lett.* 23, 3439-3443; van Boom, J.H., Grea, R., Luyten, W.C. and Vink, A.B., (1985) *Tetrahedron Lett.*, 2779-2782; Yoshikawa, M., Skuraba, M., and Kusashio, K. (1970) *Bul. Chem. Soc. Jap.*, 456-461; and Yoshikawa, M., Tetsuya, K., and Tadao, T. (1969) *Bul. Chem. Soc. Jap.*, 3505-3508.

Although enzymatic methods are widely used, they are impractical for large scale work. The presently available chemical methods also have disadvantages which make them impractical for routine use. Most of these methods are incompatible with automated synthesizers due to the instability and corrosive nature of the required reagents. In addition, low yields are frequently encountered during the phosphorylation and deblocking steps.

Description of the invention

The present invention features compositions and methods for making and using the composition for phosphorylating the 5' termini of oligonucleotides.

One embodiment of the present invention includes a composition. The composition is represented by the formula below:



wherein, if Y and Z are taken separately, each represent an alkyl, aryl, arylalkyl, cycloalkyl or cycloalkylaryl; or if Y and Z are taken together, Y and Z form an alkyl or alkylene chain with both terminal valence bonds of said chain being attached to the nitrogen atom to which Y and Z are attached; or if Y and Z are taken together, with the nitrogen atom, Y and Z form a nitrogen heterocycle including at least one additional heteroatom selected from the group consisting of nitrogen, oxygen, and sulfur. W and X are selected from the group of atomic moieties subject to nucleophilic attack or β -elimination.

Referring now to the situation where the nitrogen and Y and Z taken together form a saturated or unsaturated nitrogen heterocycle, the heterocycle includes tetrazole, indole, imidazole, benzimidazole and

similar nitrogen heterocycles some of which are characterized by at least two ethylenic double bonds which are normally conjugated. The heterocycle may include other hetero atoms such as nitrogen, sulfur, or oxygen.

Preferably, Y and Z taken separately represent alkyl moieties of three carbons or less. A preferred alkyl moiety includes isopropyl groups.

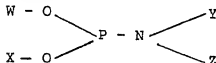
In selecting W and X, consideration should be given to the stability of the atomic moieties at room temperature in conditions that W and X will encounter during the synthesis of oligonucleotides in automated DNA synthesis machines. Such conditions include acid reagents, such as solutions of trichloroacetic acid in dichloromethane, oxidation reagents, such as iodine in water and tetrahydrofuran, acetylating reagents, such as solutions of acetic anhydride and dimethylaminopyridine, and phosphorylating reagents, such as solutions of activated nucleoside phosphoramidites.

Preferably, W and X are selected from the group consisting of alkyl, aryl, arylalkyl, cycloalkyl, cycloalkylaryl, cyanoalkyl or haloalkyl derivatives susceptible to nucleophilic attack or β -elimination.

By way of example without limitation, referring to W and X, a preferred alkyl derivative includes alkyl chains of between one and three carbons, and most preferably methyl derivatives. A preferred cycloalkyl or cycloalkylaryl derivative includes ribose derivatives of nucleotide bases and base analogs and derivatives thereof, such as 2',3'-dibenzoyluridyl derivatives. A further preferred cycloalkylaryl derivative includes fluorenylmethoxycarbonyl (Fmoc) and derivatives thereof.

By way of example without limitation, referring to W and X, preferred aryl or alkylaryl derivatives have electron withdrawing groups. Examples include halophenyl or phenol derivatives such as chlorophenyl, bromophenyl or fluorophenyl derivatives or nitrophenethyl derivatives. Preferred cyanoalkyl and haloalkyl derivatives include 2-cyanoethyl derivatives and 2,2,2-trichloro- or 2,2,2-tribromomethyl derivatives.

A further embodiment of the present inventions includes a method of phosphorylating the 5' termini of oligonucleotides. The method includes the steps of reacting the 5' termini of an oligonucleotide with a composition represented by the formula:



wherein W, X, Y, and Z are consistent with the previous description of the composition to form a reaction product. Next, oxidizing the reaction product to produce a phosphorylated compound.

After completion of the chain elongation and 5' terminal phosphorylation steps, the DNA is removed from the synthesis support and deblocked using conventional steps. If necessary, additional deblocking steps are added in order to completely remove the protecting groups. For example, in the case where ribonucleotide derivatives are used as blocking groups, the liberated oligonucleotide is then subjected to sequential treatment with sodium periodate and piperidine. The reaction with sodium periodate and piperidine removes the terminal ribonucleotides and the oligonucleotide is recovered bearing a terminal phosphate group.

The method of making the present compounds is further described in the following experimental examples which exemplify features of the preferred embodiments. The experimental protocol sets forth typical conditions and procedures.

Experimental Examples.

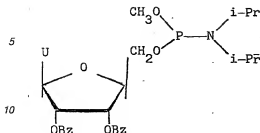
I. Synthesis Section

A. General Methods and Materials.

All chemicals were of analytical grade and used as available from manufacturers or distributors unless otherwise indicated. Unless otherwise indicated, all chemicals are available from Aldrich Chem.

B. Synthesis of 2',3'-dibenzoyluridyl-N,N-diisopropylaminomethoxyphosphine.

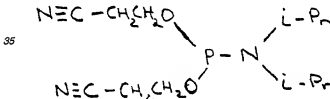
Diisopropylmethylphosphonamidic chloride (3.3g, 16.7 mmol) was added to a magnetically stirred solution of tetrahydrofuran (400 ml), diisopropylethylamine (35 ml, 200 mmol) and 2',3'-dibenzoyluridine (Sigma Chemical Co.) (11.1 mmol) at 0°C. The mixture was warmed to room temperature overnight. The hydrochloride salt formed during the reaction was removed from the reaction mixture by filtration through a sintered glass funnel. The resulting filtrate was recovered, diluted 1/1 with ethyl acetate, and washed with three 200 ml portions of 5% NaHCO₃. The organic portion was then dried over Na₂SO₄ and evaporated to yield the crude product as a yellow oil. The oil was dissolved in a mixture of dichloromethane/hexane/triethylamine (6/4/1 v/v/v) (50 ml) and applied to a column of silica gel (150 g) which had been equilibrated with the same solvent mixture. The column was eluted with the solvent mixture and fractions (10 ml) were collected. Fractions which contained the desired product were identified as 2',3'-dibenzoyluridyl-N,N-diisopropylaminomethoxyphosphine (hereinafter referred to as Compound I) represented by the formula set forth below:



wherein U is uridyl, Bz is Benzyl and i-Pr is isopropyl (Rf = 0.64 silica gel TLC developed in ethyl acetate/triethylamine 9/1). Fractions of Compound I were pooled, evaporated, and then coevaporated with toluene (3 x 5 ml) to yield the desired product as a oil (6.4 g, 94%).

C. SYNTHESIS OF BIS-(2-CYANOETHOXY)-N,N-DIISOPROPYLAMINOMETHOXYPHOSPHINE

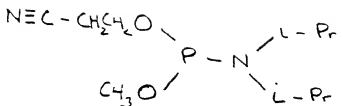
Diisopropylamino phosphorodichloridite (made in accordance with the procedure set forth in Takeo, Shimidzu, et al. Nucleic Acid Research Symposium Series No. 12,55-58 (1983)) (14.2g, 70.0 mmol) was added to a magnetically stirred solution of tetrahydrofuran (100 ml), diisopropylethylamine (36.6 ml, 210 mmol) and 3-hydroxypropionitrile (9.6 ml, 140 mmol) at 0°C. After 4 hours, the mixture was warmed to room temperature and stirred for an additional 2 hours. The hydrochloride salt was removed from the reaction mixture by filtration through a sintered glass funnel. The resulting filtrate was recovered, diluted 1/1 with ethyl acetate, and washed with three 100 ml portions of 5% NaHCO₃. The organic portion was then dried over Na₂SO₄ and evaporated to yield 17.7g of crude product as a yellow oil. The oil was dissolved in a mixture of dichloromethane/hexane/triethylamine (6/4/1 v/v/v) (50 ml) and applied to a column of silica gel (175 g) which had been equilibrated with the same solvent mixture. The column was eluted with the solvent mixture and fractions (20 ml) were collected. Fractions which contained the desired product were identified as bis-(2-cyanoethoxy)-N,N-diisopropylaminomethoxyphosphine (hereinafter referred to as Compound II) represented by formula set forth below:



wherein i-Pr is isopropyl. (Rf = 0.60 silica gel TLC developed in ethyl acetate/triethylamine 9/1). Fractions containing Component II were pooled, evaporated, and then coevaporated with toluene (3 x 5 ml) to yield the desired product as a colorless oil (11.1g, 58.7%) ³¹P NMR = -146.75 ppm, relative to trimethylphosphate in CH₂Cl₂.

D. SYNTHESIS OF 2-CYANOETHOXYMETHOXY-N,N-DIISOPROPYLAMINOPHOSPHINE

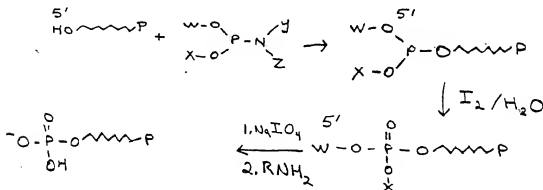
Diisopropylmethyl phosphoramidite chloride (10.7 g, 52.5 mmol) was added to a magnetically stirred solution of THF (100 ml), diisopropylethylamine (27.5 ml, 157.5 mmol) and 3-hydroxypropionitrile (7.2 ml, 105 mmol) at 0°C. After 4 hours, the mixture was warmed to room temperature and stirred for an additional 2 hours. The hydrochloride salt was removed from the reaction mixture by filtration through a sintered glass funnel. The resulting filtrate was recovered, diluted 1/1 with ethyl acetate, and washed with three 100 ml portions of 5% NaHCO₃. The organic portion was then dried over Na₂SO₄ and evaporated to yield the crude product which was dissolved in a mixture of dichloromethane/hexane/triethylamine (6/4/1 v/v/v) (50 ml) and applied to a column of silica gel (175 g) which had been equilibrated with the same solvent mixture. The column was eluted with the solvent mixture and fractions (20 ml) were collected. Fractions which contained the desired product were identified as 2-cyanoethoxymethoxy-N,N-diisopropylaminophosphine (hereinafter referred to as Compound III) represented by the formula set forth below:



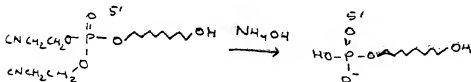
wherein i-Pr is isopropyl. Fractions containing Compound III were pooled, evaporated, and then coevaporated with toluene (3 x 5 ml).

E. SYNTHESIS OF OLIGONUCLEOTIDES HAVING 5' TERMINAL PHOSPHATE MONOESTERS

In a typical synthesis, the synthesizer is programmed to permit the addition of the modified phosphoramidite, such as compounds I, II and III, as they would any other phosphoramidite compound. After completion of the chain elongation steps and 5' terminal phosphorylation steps, the DNA is removed from the synthesis support and deblocked in the usual manner. To deblock the terminal phosphate triester and remove additional blocking groups, referring now to ribonucleotide blocking groups and related compounds, the liberated oligonucleotide is then subjected to a sequence of appropriate deblocking steps. For example, in the case of ribonucleotide blocking groups deblocking is accomplished with a sequential treatment with sodium periodate and piperidine as outlined below.



Deprotection of oligonucleotides coupled with phosphorylating agents such as compound II and related compounds can be achieved during the base deblocking step with ammonium hydroxide as set forth below:



The compound 2-cyanoethoxymethoxy-N,N-diisopropylaminophosphine, does not efficiently provide the desired oligonucleotide phosphate-monoester. Complete removal of the protecting groups is difficult under normal conditions to which DNA can be subjected to.

Several fragments containing the synthetic 5' phosphate group have been prepared. Figures 1 and 2 illustrate the HPLC characterization data which compares the synthetically phosphorylated DNA with standard samples obtained from commercial sources. Turning first to Figure 1, Plot A describes HPLC characterization data for a commercially obtained standard sample of pT₁₂. Plot B describes HPLC characterization data for a sample of pT₁₂ after synthesis with Compound II. Plot C describes HPLC characterization data for a coinjection of a standard pT₁₂ sample with a pT₁₂ sample obtained using Compound II.

Turning now to figure 2, Plot A describes HPLC characterization data for a commercial standard sample of pT₁₂. Plot B describes HPLC characterization data for a sample of synthetic UpT(pT)₁₁. Plot C describes HPLC characterization data for a mixture containing synthetic UpT(pT)₁₁ and pT₁₂ obtained after the periodate oxidation of UpT(pT)₁₁. Plot D is (pT)₁₂ obtained after periodate treatment of Up(pT). Plot E describes HPLC

characterization data for a commercial standard sample of (pT)₁₂. Finally, Plot F describes HPLC characterization data for the coinjection of a commercial standard sample of (pT)₁₂ and (pT)₁₂ obtained after oxidation of UpT (pT)₁₁.

Turning now to Figure 3, Plot A describes HPLC retention times for a commercial standard sample of (pT)₁₂. Plot B describes HPLC retention time data for 5'EDApT(pT)₁₁ obtained from (pT)₁₂ synthesized using Compound II. Plot C describes HPLC retention time data for a coinjection of EDApT(pT)₁₁ obtained from a commercially obtained sample (pT)₁₂ and 5'EDApT(pT)₁₁ obtained from (pT)₁₂ synthesized using Compound II. Plot D describes HPLC retention time data for a commercial standard of (pT)₁₂. Finally, Plot E describes HPLC retention times for 5' EDApT(pT)₁₂ obtained from (pT)₁₂ synthesized using Compound I.

Short fragments of DNA bearing the synthetic phosphate group have been combined enzymatically to produce fragments of extended length. It should be noted, that Compound III does not favor complete removal of the phosphate protecting groups. Both of the protecting groups of Compound III are not susceptible to β -elimination which appears to be a preferred method of removal.

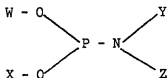
The present methods and compounds offer improvements to synthetic DNA which can be directly synthesized bearing a 5' phosphate group. The present methods and compounds are compatible with automated DNA synthesizers and give products of high yield. The ability to directly isolate 5' phosphorylated DNA simplifies procedures required to construct artificial genes of commercial significance. The need for enzymatic phosphorylation is eliminated and the total time required to build synthetic DNA having extended length has been reduced. Synthetic fragments bearing 5' terminal phosphate groups can also be used to synthesize DNA bearing terminal amine functional groups. These fragments can then undergo subsequent modification to prepare DNA bearing nonradioactive reporter groups.

Thus, while preferred embodiments of the invention has been described, the present invention is capable of variation and modification and, therefore, the invention should not be limited to the precise details set forth, but should include such changes and alterations that fall within the purview of the following claims.

Any alkyl groups referred to herein (including cycloalkyl groups) preferably contain not more than 12, most preferably not more than 6 carbon atoms. "Aryl" refers to any aromatic nucleus, e.g. a benzene or naphthalene nucleus.

Claims

1. A method of phosphorylating a 5' terminus of an oligonucleotide, including the steps of
 - a) reacting the 5' terminus of the oligonucleotide with a compound of the formula



wherein Y and Z taken separately each represents alkyl, aryl, arylalkyl, cycloalkyl, or cycloalkylaryl; or Y and Z taken together form a saturated or unsaturated hydrocarbyl chain, preferably an alkyl or alkylene chain, with both terminal valence of said chain being attached to the nitrogen atom to which Y and Z are attached; or Y and Z taken together with the nitrogen atom form a saturated nitrogen heterocycle including at least one additional heteroatom selected from nitrogen, oxygen, and sulfur; and W and X are blocking groups, preferably selected from groups of atomic moieties subject to nucleophilic attack or β -elimination; and

- b) removing the blocking groups W and X.

2. A method according to Claim 1 wherein Y and Z taken separately represent alkyl moieties of three carbons or less, or when Y and Z together form a saturated or unsaturated hydrocarbyl chain, said chain contains up to 5 carbon atoms.

3. A method according to Claim 2 wherein the alkyl moieties represent isopropyl groups.

4. A method according to any preceding claim wherein W and X are stable at room temperature and are stable in acid reagents, oxidizing reagents, acetylating reagents and phosphorylating reagents.

5. A method according to any preceding claim wherein W and X are selected from alkyl, aryl, arylalkyl, cycloalkyl, cycloalkylaryl, cyanoalkyl or haloalkyl derivatives capable of subject to nucleophilic attack or β -elimination.

6. A method according to any of Claims 1 to 3 wherein said blocking groups susceptible to nucleophilic attack include alkyl derivatives having three carbons or less and phenyl derivatives having electron withdrawing groups.

7. A method according to Claim 6 wherein said alkyl derivative is methyl.

8. A method according to Claim 6 wherein said phenyl derivative is selected from chlorophenyl, bromophenyl, and fluorophenyl derivatives.

0266168

FIG.1A

28.4 min

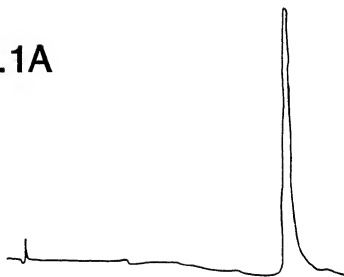


FIG.1B

29.4 min

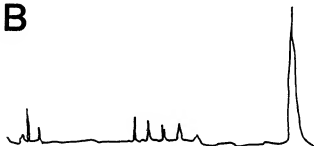
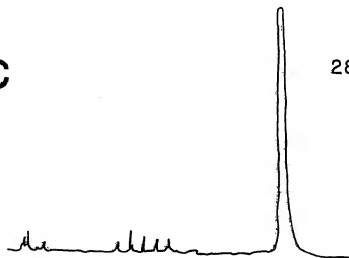


FIG.1C

28.2 min



0266168

FIG.2A

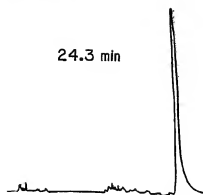


FIG.2D

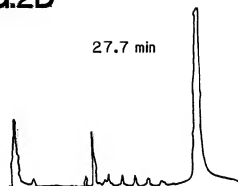


FIG.2B

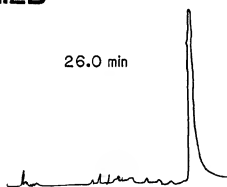


FIG.2E



FIG.2C

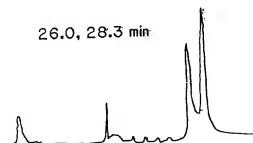
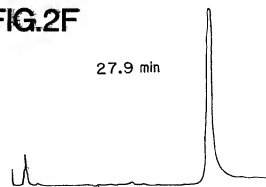


FIG.2F



0266168

FIG.3A

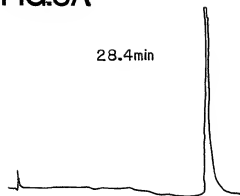


FIG.3D

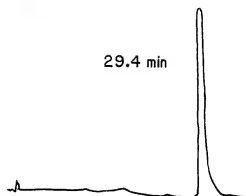


FIG.3B

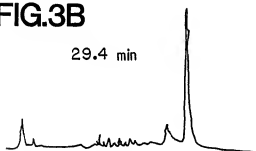


FIG.3E

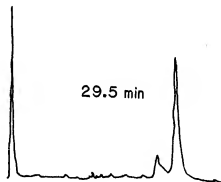
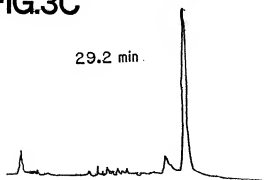


FIG.3C



(12) **EUROPEAN PATENT APPLICATION**

(21) Application number: 87309479.1

(51) Int. Cl.5: **C07H 21/04**

(22) Date of filing: 27.10.87

(23) Priority: 31.10.86 US 926010

(43) Date of publication of application:
04.05.88 Bulletin 88/18

(54) Designated Contracting States:
AT BE CH DE ES FR GB IT LI LU NL SE

(40) Date of deferred publication of the search report:
03.10.90 Bulletin 90/40

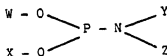
(71) Applicant: **AMOCO CORPORATION**
200 East Randolph Drive
Chicago Illinois 60601(US)

(72) Inventor: **Goody, Edward Patrick**
249 Wilson Street
Marlboro Massachusetts 01752(US)

(74) Representative: **Ritter, Stephen David et al**
Mathys & Squire 10 Fleet Street
London EC4Y 1AY(GB)

(52) **Compositions and methods for the synthesis of oligonucleotides having 5'-phosphorylated termini.**

(57) Compounds of formula



are provided for use in methods for the chemical 5'-phosphorylation of oligonucleotides for DNA phosphoramidite synthesis.



DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
X	EP-A-0 090 789 (MONSATO CO.) * Whole document *	1-19	C 07 H 21/04
X	EP-A-0 196 101 (CHIRON CORP.) * Example 7; claims *	1-18	
X	WO-A-8 304 028 (APPLIED MOLECULAR GENETICS) * Example 1; claims *	1-2,4,6 -7,10- 12,17- 18	
X	EP-A-0 035 719 (UNIVERSITY PATENTS INC.) * Whole document *	1,4,10- 11,14, 17-18	C 07 H 21/00
X	EP-A-0 195 277 (HOECHST AG) * Whole document *	1-19	
X	TETRAHEDRON LETTERS, vol. 24, no. 3, 1983, pages 245-248, Pergamon Press Ltd, GB; L.J. MCBRIDE et al.: "An investigation of several deoxynucleoside phosphoramidites" * Whole document *	1-4,6,7 10-12, 14,17, 18	
X	WO-A-8 404 749 (BECKMAN INSTRUMENTS INC.) * Page 18, compound IVa; claims *	1,4,6,7 10,11, 14,17, 18	C 07 H 21/00
X	EP-A-0 119 621 (HOFFMAN-LA ROCHE & CO.) * Figure 11 *	1,2,4,6 7,10, 11,14, 17,18	
	---	-/-	
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 16-07-1990	Examiner WATCHORN P.W.
CATEGORY OF CITED DOCUMENTS			
<p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : oral-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited to the application L : document cited for other reasons</p> <p>..... & : member of the same patent family, corresponding document</p>			



DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
X	EP-A-0 108 387 (HOFFMAN-LA ROCHE & CO.) * Figure 9 *	1, 2, 4, 6 7, 10, 11, 14, 17, 18	

P, X	EP-A-0 216 357 (NIPPON ZEON CO., LTD) * Whole document *	1-5, 9- 12, 17, 18	

P, X	EP-A-0 235 301 (TEIJIN LTD) * Figure 6 *	1-4, 17, 18	

P, X	WO-A-8 607 362 (UNIVERSITY PATENTS INC.) * Page 3; page 12; claims *	1, 17, 18	

			TECHNICAL FIELDS SEARCHED (Int. Cl. 4)
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 16-07-1990	Examiner WATCHORN P. W.
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			